Alloimmunization to the D antigen by a patient with weak D type 21

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Antibodies of apparent D specificity may be found in D+ patients. We report a D+, multi-transfused Caucasian woman with myelodysplasia who exhibited several alloantibodies. One antibody was a moderately strong (2+) anti-D that persisted for 9 months, until the woman died. Molecular analysis of the patient's RHD gene identified the rare weak D type 21 (938C>T) allele. D alloantibodies do not occur in patients with most weak D types, but some patients with a weak D phenotype, including those with type 21, can produce antibodies to nonself epitopes of the wild-type D antigen. ***Immunohematology* 2010;26:27–29.**

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Antibodies with D specificity are unusual in D+ patients. Alloantibodies to D may be (1) passively transfused in donor plasma products, (2) passively transferred but actively produced by lymphocytes carried in transplanted organs, and (3) actively produced by an alloimmunized patient. Autoantibodies to D may be warm- or cold-reactive antibodies with a preference for D+ RBCs or may be antibodies to LW that mimic anti-D specificity.[1]

In practice, the combination of clinical, serologic, and molecular investigations helps to determine why a D+ patient has anti-D. Establishing the source of a D+ patient's alloantibody or autoantibody with D specificity is important in deciding whether D+ or D– units should be transfused to optimize donor RBC survival (Table 1).

In this case report, we describe a woman whose RBCs were strongly D+ at the phenotypic level, but had an *RHD* genotype consistent with weak D, and an alloanti-D.

Table 1. Evaluation and transfusion of patients with D+ RBCs and serum anti-D

Etiology/ pathogenesis	Diagnostic evidence	Preferred RBC transfusion
Passive alloantibody	History of transfusion with D– RBC, plasma, IVIG, RhIG, other product with anti-D	D– until anti-D wanes
Alloantibodies from passenger lymphocytes in donor organ	Organ transplant from D– donor to D+ recipient	D– until anti-D wanes
Autoantibody to D antigen	Positive direct/indirect antiglobulin test (anti-IgG) Anti-D specificity in eluate or serum History: pregnancy, warm autoimmune hemolytic anemia, malignancy	D– over D+
Autoantibodies or alloantibodies to LW	Antibody fails to react with RBC treated with dithiothreitol Antibody is absorbed by D– RBC	D– over D+
Alloantibody to epitope absent from normal D protein	Anti-D fails to react with RBC of person who produced it Anti-D fails to react with D– RBC	D– only

Case Report

A 72-year-old, gravida 3, Caucasian woman with myelodysplasia and pancytopenia was transferred from another hospital, where in 6 months of treatment, she had received an unknown number of platelet transfusions, but no RBC transfusions.

In our hospital and before any RBC transfusion, the patient's RBCs serologically typed as D+ (4+) by tube agglutination at immediate spin phase, using a monoclonal-polyclonal blended anti-D (Ortho Clinical Diagnostics Co., Raritan, NJ). During a disease course of 13 months, the patient received 37 apheresis-processed platelet units and 108 leukocyte-reduced units of RBCs, which produced alloimmunization to K, E, and C^w. After 9 months, her serum also demonstrated a 2+ anti-D in a gel-based, antibody detection system (Micro Typing Systems, Inc., Ortho Clinical Diagnostics, Pompano Beach, FL). The initial DAT (tube, anti-IgG) and all but one subsequent DAT were negative as was the auto-control. On the one occasion, the DAT became weakly positive in a mixed-field reaction. Acid eluates prepared from the patient's RBCs contained only anti-E. There was no history of organ transplantation or administration of a plasma product that might contain anti-D. The anti-D persisted throughout the patient's course. Blood samples were collected and referred for *RHD* and *RHCE* gene analyses.

Although continued transfusions with D– blood were prescribed, the patient refused all further medical treatment. Her myelodysplasia progressed to early acute leukemia before she expired at home.

Molecular Test Results

Molecular tests were performed at the Molecular Red Cell and Platelet Testing Laboratory of the American Red Cross, Penn-Jersey Region, and the University of Pennsylvania DNA Sequencing Facility, both in Philadelphia,

Pennsylvania. The patient was found to be heterozygous for *RHD*. No changes associated with any of the known partial D phenotypes were identified. A change (938C>T) was identified that is associated with weak D type 21 that is predicted to encode an intracellular amino acid change of Pro313Leu. The patient's RH genotype could be interpreted to be *RHD*weak D type 21, RHCE*Ce/RHCE*ce*.

Discussion

Initially, the finding of anti-D in a D+ patient was puzzling and the several causes listed in Table 1 were considered. Patients whose anti-D was passively transferred in plasma products (IVIG, RhIG, etc.) or whose serum antibodies were produced by a donor's passenger lymphocytes may require D– RBCs until the acquired anti-D has weakened, usually in a matter of weeks to months.

Passive anti-D, however, was ruled out by history. Before hospital admission at our institution, the patient had been transfused only with apheresis platelets, but she had not been transfused with plasma or cellular products from D– donors, nor had she been infused with IVIG or injected with RhIG. Persistence of the anti-D was further evidence that the anti-D did not involve passive immunization.

The transfer of passenger lymphocytes could be excluded by history as well. The patient had never received a transplant. Passenger lymphocytes are alloantibody-secreting B cells that are carried in a transplanted donor organ to a recipient. An anti-D produced by passenger lymphocytes arises in the donor by alloimmunization to the D antigen before transplantation. Donor lymphocytes continue to manufacture detectable anti-D for about 3 months after transplantation.

Many patients with autoimmune hemolytic anemia and mimicking antibodies to Rh antigens are treated medically and transfusion is avoided altogether. In other patients, transfusion can be delayed until the concentration of antibodies is reduced by medical treatment. Many mimicking warm autoantibodies have apparent Rh specificity, especially anti-e. Only rare D+ patients will possess warm autoantibodies that mimic anti-D. If a patient with autoantibodies requires transfusion, antigen-negative donor RBCs may be preferable because they may survive longer than antigen-positive RBCs.[2]

Antibodies to LW are almost always autoantibodies; those that are less potent appear to have anti-D specificity because the number of D antigen sites per RBC correlates with the number of LW antigen sites.[3] Thus, D– RBCs, which have fewer LW antigen sites than D+ RBCs, may not be agglutinated by an anti-LW. However, anti-LW may be distinguished from anti-D by its failure to agglutinate RBCs treated with DTT. If transfusion is required for a patient with autoanti-LW, D– RBCs are preferable to D+ RBCs providing such a patient is c+.

Patient autoantibodies, including anti-LW, are often transient, but our patient's anti-D persisted for 4 months at constant serologic strength (2+ by the IgG gel test). Alloantibodies to Rh antigens are often durable (85%). Negative DATs and absence of RBC agglutination in autocontrol tests also indicated the presence of D alloantibody.

LW alloantibodies, which can mimic anti-D, arise only in very rare individuals whose RBCs are LW(a–b–) or LW(a–b+). Neither autoantibodies nor alloantibodies to LW were specifically evaluated in the patient described here.

Alloantibodies to the D antigen occur in people who are known to lack one or more epitopes of D (partial D phenotype). On the other hand, anti-D alloimmune responses in patients with other weak D types are unusual because their RBCs do not appear to lack D epitopes when tested with a standard battery of anti-D monoclonal reagents.[4]

Suspicion that the patient had been alloimmunized to D antigen was raised by the serologic finding of negative DATs and the antibody's persistence. In addition, people who carry variants of the D antigen that are detectable only by molecular means are capable of producing antibodies to wild-type protein. In the present case, finding a variant *RHD* gene supported the clinical and serologic evidence of D alloimmunization.

Historically, some D+ individuals who made anti-D were initially considered to express a weak D phenotype (first termed "D^U") when tested with human polyclonal reagents. Serologic evidence of alloimmunization to D in a D+ person included a negative DAT, a serum antibody, and, for some partial D phenotypes, the patient's RBCs also expressed a low-prevalence Rh antigen.[5] A D+ patient with alloanti-D was thought to lack part(s) of the D antigen, i.e., a "partial D" type. A person with a partial D phenotype would only produce antibody to those epitopes of the D antigen missing from his or her own RBCs. Investigative use of monoclonal antibody revealed many epitopes of the D antigen and supported the concept that partial D types lacked one or more epitopes of their D antigen. The monoclonal antibodies also extended the identification and classification of the various partial D types initially defined by polyclonal anti-D.

Many individuals, however, were found to have RBCs that agglutinated poorly with common anti-D reagents, but demonstrated the presence of all known epitopes. These D+ RBCs were called "weak D" in preference to D^U. Weak D RBCs expressed fewer D molecules per RBC, a finding that accounted for their weak agglutination with reagent antibodies. Theoretically, individuals who carried these weak D types should not be able to develop alloantibodies to D. However, it is virtually impossible to distinguish a person with a weak D phenotype from a person with a weak partial D phenotype by serologic tests; only the production of alloanti-D will reveal someone to have a weak partial D phenotype.

When molecular analysis became available, the *RHD* (and *RHCE*) gene(s) revealed far more extensive and complex polymorphisms than were evident by serologic methods. Weakly agglutinating D serotypes could be related to point mutations, nonsense mutations, deletions, and tandem

rearrangements in the *RHD* and *RHCE* DNA. The cause of weak RBC agglutination was confirmed to be an absence of epitopes in partial D types, a decreased number of protein molecules in weak D types, or both. In addition, partial D phenotypes are expressions of genotypes that encode for changes in the amino acids on the outer surface of the RBC where the D protein loops outside the RBC membrane. Patients whose RBCs express partial D types are often immunized to D+ RBCs by transfusion or pregnancy. On the other hand, genotypes that encode weak D phenotypes are predicted to result in intracellular or intramembranous changes in amino acids that, in theory, express all epitopes of D so that patients whose RBCs express weak D types are not expected to produce anti-D after immunization by transfusion or pregnancy. Theory is partly supported by evidence—the most common weak D variants (types 1–3), which constitute 70 to 90 percent of all weak D variants, do not produce alloanti-D.[6]

Molecular examination of the various partial and weak D types indicated that many serologic phenotypes had more than one basis, but did not suggest that new epitopes could be identified.[7] However, serologic evidence suggested otherwise. Anti-D has been found in some weak D patients whose D protein is predicted by their DNA sequences to have altered intramembranous and intracellular amino acids of the D protein. Thus, some weak D molecular alleles contain missense mutations that not only reduce the number of antigens per RBC and cause weak RBC agglutination by reagent antibodies but also cause a loss of D epitopes and produce changes in the three-dimensional antigenic structure of the D protein.

The case presented here indicates that the rare weak D type 21 is an allelic variant whose patient-carriers are susceptible to transfusion alloimmunization by wild-type D antigen. Weak D type 21 is the result of a point mutation that alters an amino acid thought to be in an intracellular region of the D protein.[8] The rare allele was first discovered in one individual during investigation of 270 known weak D samples in people from northern Germany,[9] and there has been one subsequent independent observation of it in Austria.[10] In both cases, the allele has been found on the same chromosome *(cis)* as a *RHCE*Ce* allele in the haplotype *RHD*$^{weak\ D\ type\ 2}$*-RHCE*Ce*. The Pro313Leu change in the translated D protein is in its tenth intracellular helix, and all 37 tested epitopes of the D antigen appear to be present.[9] As expected, the number of D antigen sites per RBC is reduced (5.2×10^3) when compared with the normal number (10^4 to 2.5×10^4) of sites.[11] This antigen density is the highest among all known weak D alleles. The high density probably accounts for the patient's 4+ serotype and explains why she was not identified as a weak D carrier.

Summary

We evaluated a patient with a strongly expressed D antigen with a posttransfusion antibody to the D antigen. The antibody had clinical and serologic characteristics of an alloantibody. After RHD and RHCE genotyping, the patient was found to have the *RHD*$^{weakD\ type\ 21}$*RHCE*Ce/RHCE*ce* genotype ($R_1^{weak\ D\ type\ 21}$r phenotype). Although most patients with a weak D phenotype do not produce alloanti-D, patients who carry the rare weak D type 21 in the absence of a normal *RHD* allele appear susceptible to the production of D alloantibodies.

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